Bacterial protease activity, an indicator of bacterial pathogenicity in chronic wounds even in the absence of overt clinical signs

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Aims

To assess levels of Bacterial Protease Activity (BPA) in chronic wounds and how the occurrence of BPA relates to clinical signs of infection.

Background/Introduction

Increased bacterial bioburden in chronic wounds has been reported to impair wound healing and can lead to systemic infection. However, some chronic wounds fail to exhibit the classic signs of infection[1,2]. As a result, clinical examination can under-diagnose infections in chronic wounds. Moreover, current culture techniques have limited reliability on their own and can lead to the over diagnosis of infection. Bacterial proteases are a type of virulence factor, present when bacteria are in a pathogenic state[3].

A number of known proteases have been characterised from some of the frequently reported bacteria in chronic wounds[4,5]. The impact of bacterial proteases has been documented in a range of acute and chronic medical conditions, including cystic fibrosis, eye infections and wound infections[5,6]. Therefore, BPA may be a useful method of detecting the presence of pathogenic bacteria in chronic wounds.

Results – Clinical Signs of Infection and Bacterial Protease Activity (BPA)

The wound types were distributed across a range of chronic wounds (Figure 1). Just under 20% of all wound swabbed exhibited Clinical Signs of infection (Figure 2). In contrast, 47% of all wounds swabbed were 'positive' for BPA (Figure 3); of these, 72% had no Clinical Signs (Figure 4). We suspect that these wounds had pathogenic bacteria but had not progressed to overt infection as the majority of these samples had ≥10(5) CFU (Figure 5).

Figure 1 - Distribution of Wound Types (n=186)

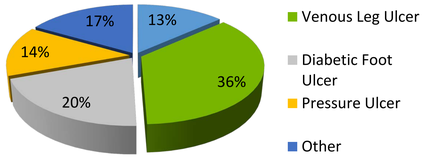


Figure 2 – Wounds with and without Clinical Signs

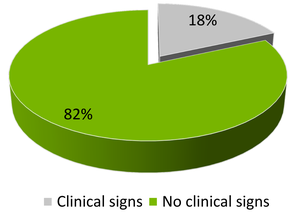


Figure 3 - BPA Assessment

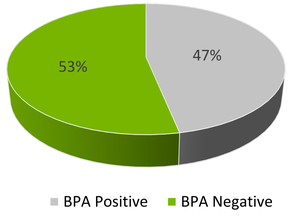


Figure 4 - BPA positive and Clinical Signs analysis

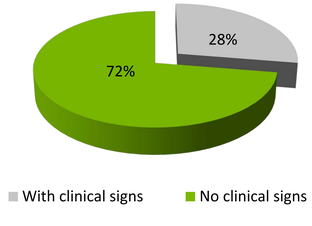
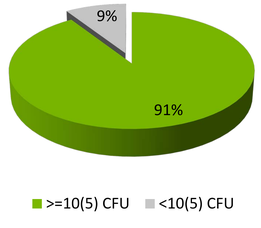


Figure 5 - Analysis of elevated BPA only and CFU dataset



Methods

186 patients with chronic wounds from 4 wound care centres in geographically distinct regions of the United States underwent assessment for the signs of critical colonisation / infection ("Clinical Signs") using validated assessment criteria[7,8]. The wounds were swabbed to assess BPA levels and bioburden (CFU), based on a quantitative swab.

BPA was assessed using a quantitative laboratory fluorometric assay for Casein which had been modified using an inhibitor to exclude potential effects of the predominant host protease activity in the sample – Human Neutrophil Elastase (HNE) – on the test result.

Based on the data obtained, the level of BPA associated with clinical signs of infection was established. Chronic wounds with these levels of BPA or above were considered 'positive' for BPA and indicative of the presence of pathogenic bacteria.

Results – Bioburden

The majority of wounds analysed, approximately 80%, had ≥10(5) CFU (Figure 6), currently the most frequently cited threshold for infection.
Further analysis of the whole dataset (n=186) showed that almost as many samples with CFU≥10(5) were 'positive' for BPA (42%) as were 'negative' for BPA (39%) (Figure 7).

Figure 6 - CFU analysis

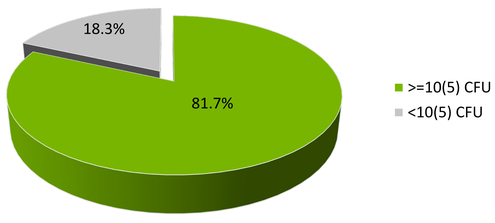
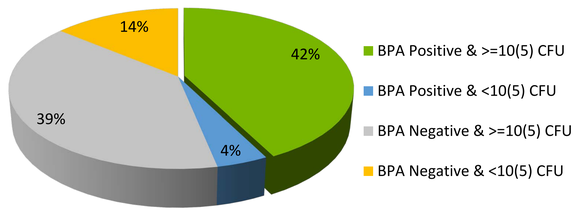


Figure 7 - Analysis of BPA and CFU (total data set)



Conclusions/Discussion

- Bacterial proteases are a type of virulence factor, which have been implicated in a range of medical conditions, including wound infections.
- Clinical Signs of critical colonisation/infection may not be apparent in all chronic wounds.
- Measuring CFU levels only in chronic wounds does not determine if bacteria are in a pathogenic state.
- Testing wound fluid for bacterial protease activity may be a useful method for detecting the presence of pathogenic bacteria, at a clinically significant stage in the infection continuum.
- On this basis, a new qualitative point of care test is under development to detect bacterial protease activity in chronic wounds, which may help identify the presence of pathogenic bacteria.

References
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